Allergic Contact Sensitizing Chemicals as Environmental Carcinogens

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Chemicals that were bioassayed by the National Toxicology Program (NTP) and that also produce allergic dermatitis (ACD) in humans were evaluated for their tumorigenic characteristics. The impetus for the study was that most contact sensitizers, i.e., those that produce ACD, and genotoxic carcinogens are chemically similar in that they are electrophilic, thereby producing adducts on macromolecules including protein and DNA. This similarity in chemical behavior suggests that many contact sensitizers might be environmental carcinogens. All of the published NTP bioassays by early 1996 that had both genotoxicity and carcinogenicity studies were included in this analysis. The NTP chemicals had been chosen for bioassay without regard to their ability to produce ACD. Of the 209 chemicals that were bioassayed, there were 36 (17%) that were known to be human contact sensitizers; about half of these were positive on tumor bioassays. The contact sensitizers differed from the NTP sample as a whole by having a proportionately larger number of nongenotoxic chemicals by the Ames *Salmonella* assay, presumably because more of them were selected on the basis of widespread usage rather than structural resemblance to known carcinogens. Compared to the nongenotoxic chemicals, the genotoxics were stronger carcinogens in that they had a higher incidence of positive tumor bioassays, with twice the number of organs in which tumors were induced. The nongenotoxic chemicals had a preference for tumor induction in parenchymal tissues in contrast to epithelial tissues. The contact sensitizers showed essentially the same characteristics as the whole NTP sample when stratified according to genotoxicity. Judging by the chemicals that were chosen primarily for their widespread use rather than for their structural resemblance to carcinogens, the addition of a test for contact sensitization to the Ames test as a screening tool would increase the tumorigenic detection efficiency by about 40% because of the nongenotoxic tumorigens. A ballpark estimate suggests that there could be several thousand contact sensitizers for humans in commercial use that are rodent tumorigens. *Key words*: allergic contact dermatitis, cancer, carcinogens, contact sensitization, immunology, National Toxicology Program.

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There is a possibility that many of the hundreds of chemicals that produce allergic contact dermatitis (ACD) in humans might be environmental carcinogens (*1*). Most contact sensitizers are electrophiles (*2*). They are able to attach themselves as adducts on macromolecules, specifically proteins, as the basis for the induction of ACD. Electrophilicity is also a common characteristic of genotoxic carcinogens (*3*); adducts form on DNA as well as on protein, resulting in genetic damage and the initiation of the carcinogenic process. Genotoxic carcinogens have been shown to be contact sensitizers in the skin of the guinea pig (*4*) and in the mouse ear (*5*). A number of chemicals known to be contact sensitizers for humans (*6*) are found in the NIH list of carcinogens (*7*); these include nickel, chromium, benzidine, beryllium, cadmium, coal tar, epichlorohydrin, butyl hydroxyanisole, DDT, *p*-dichlorobenzene, dimethylaminoazobenzene, formaldehyde, hydrazine, lindane, 4,4′-methylenedianaline, thiourea, and toluene 2,4-diisocyanate. Hence, carcinogens can be contact sensitizers and contact sensitizers can be carcinogens. While contact sensitization is a skin response, it is clear from the above examples that cancer induction by agents that are contact sensitizers can occur internally by a variety of exposure routes not involving the skin.

Given the possibility that contact sensitization might be a practical means of identifying previously unsuspected environmental carcinogens, we wondered what pattern of tumorigenic responses would be obtained if a sample of the many hundreds of known human contact sensitizers were subjected to conventional genotoxicity and rodent cancer bioassays. As an indirect approach to the question, we examined the NTP carcinogenesis and genotoxicity bioassay series for the presence of contact sensitizers and their tumorigenic effects.

The NTP series encompasses hundreds of chemicals that have been subjected to highly standardized and carefully monitored bioassays. The chemicals were recommended for study mainly by federal health and environmental agencies without regard to their ability to produce ACD. We examined several hundred NTP bioassays and found that they included a number of chemicals, both genotoxic and nongenotoxic, that are known to be human contact sensitizers. The essential finding was that

Table 1. SAL^+,CHO^+ NTP compounds

Chemical	CAS	CS	RFB[a]	Tumor score[b]	Route
Allyl glycidyl ether	106-92-3	+	2	1se	Inhalation
2-Aminoanthroquinone	117-79-3		2	4	Feed
1-Amino-2-methylanthraquinone	082-28-0		2	4	Feed
2-Amino-4-nitrophenol	099-57-0		2	1se	Gavage
2-Amino-5-nitrophenol	121-88-0		2	1se	Gavage
4-Amino-2-nitrophenol	119-34-6		2	1	Feed
2-Amino-5-nitrothiazole	121-66-4		2	2	Feed
5-Azacytidine	320-67-2		5	(1)	Intraperitoneal
Azinphosmethyl	086-50-0		1	0	Feed
p-Benzoquinone dioxime	105-11-3		1	1	Feed
1,2,3-Benzotriazole	095-14-7	+	1	0	Feed
2-Biphenylamine hydrochloride	2185-92-4		2	1	Feed
Bis(2-chloro-1-methylethyl) ether	108-60-1		2	(4)	Gavage
Captan	133-06-2	+	1	2	Feed
Chlorinated trisodium phosphate	56802-99-4		1	0	Gavage
4-(Chloroacetyl)-acetanalide	140-49-8		2	0	Feed
p-Chloroanaline	106-47-8		2	0	Gavage
2-Chloroethanol	107-07-3		2	0	Dermal
2-Chloromethylpyridine hydrochloride	6959-47-3		2	0	Gavage
3-Chloromethylpyridine hydrochloride	6959-48-4		2	3	Gavage
4-Chloro-*m*-phenylenediamine	5131-60-2	+	2	2	Feed
4-Chloro-*o*-phenylenediamine	95-83-0		2	6	Feed
2-Chloro-*p*-phenylenediamine	61702-44-1		2	0	Feed
Chloropicrin	76-06-2		1	(0)	Gavage

(*continued on next page*)

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Table 1. (*continued*)

Chemical	CAS	CS	RFB[a]	Tumor score[b]	Route
C.I. Acid orange 3	6373-74-6	+	2	1	Gavage
C.I. Disperse blue 1	2475-45-8	+	2	2	Feed
Coumarin	91-64-5	+	1	4	Gavage
m-Cresidine	102-50-1		2	(2)	Gavage
p-Cresidine	120-71-8		2	8	Feed
Cupferron	135-20-6		2	12	Feed
Cytembena	21739-91-3		5	2	Intraperitoneal
2,4-Diaminoanisole sulfate	615-05-4		2	6	Feed
2,4-Diaminotoluene	90-80-7		7	4	Feed
1,2-Dibromo-3-chloropropane	96-12-8		7	9	Inhalation
1,2-Dibromoethane	106-93-4		7	13	Inhalation
2,3-Dibromo-1-propanol	96-13-9		4	30	Dermal
1,2-Dichloroethane	107-06-2		4	8	Gavage
2,6-Dichloro-*p*-phenylenediamine	609-20-1		2	2	Feed
1,2-Dichloropropane	78-87-5		2	2se	Gavage
Dichlorvos	62-73-7		2	4	Gavage
Diglycidyl resorcinol ether	101-90-6		3	4	Gavage
Dimethoxane	828-00-2		7	0	Gavage
2,4-Dimethoxyaniline hydrochloride	54150-69-5		2	0	Feed
3,3′-Dimethoxybenzidine-4,4′ diisocyanate	91-93-0		2	8	Gavage, feed
Dimethyl hydrogen phosphite	868-85-9		8	2	Gavage
1,2-Epoxybutane	106-88-7		2	4	Inhalation
Glycidol	556-52-5		2	24	Gavage
HC Blue 1	2784-94-3		2	3	Feed
HC Red 3	2871-01-4		2	0	Gavage
Hydrazobenzene	122-66-7		2	5	Feed
8-Hydroxyquinoline	148-24-3		2	0	Feed
Iodinated glycerol	5634-39-9		2	4se	Gavage
Lasiocarpine	303-34-4		7	(5)	Feed
4,4′-Methylenedianaline	13552-44-8		2	10	Water
2-Methyl-1-nitroanthraquinone	129-15-7		2	4	Feed
Michler's ketone	90-94-8		2	4	Feed
1,5-Napthalenediamine	2243-62-1		2	6	Feed
N-(1-Napthyl)ethylenediamine dihydrochloride	1465-25-4		2	0	Feed
5-Nitroacenaphthene	602-87-9		2	8	Feed
p-Nitroanaline	100-01-6		2	0	Gavage
o-Nitroanisole	91-23-6		2	10	Feed
4-Nitroanthranilic acid	619-17-0		2	0	Feed
6-Nitrobenzimidazole	94-52-0		2	2	Feed
p-Nitrobenzoic acid	62-23-7		2	1	Feed
Nitrofurantoin	67-20-9		2	6	Feed
Nitrofurazone	59-87-0		4	3	Feed
1-Nitronapthalene	86-57-7		2	0	Feed
2-Nitro-*p*-phenylenediamine	5307-14-2		2	1	Feed
3-Nitroproprionic acid	504-88-1		1	2	Gavage
p-Nitrosodiphenylamine	156-10-5		2	2	Feed
5-Nitro-*o*-toluidine	99-55-8		2	4	Feed
4,4′-Oxydianiline	101-80-4		2	9	Feed
Phenazopyridine hydrochloride	136-40-3		5	3	Feed
Phenoxybenzamine hydrochloride	63-92-3		5	4	Intraperitoneal
p-Phenylenediamine dihydrochloride	624-18-0	+	2	0	Feed
1,2-Propylene oxide	75-56-9		2	4	Inhalation
Quercetin	117-39-5		3	1se	Feed
Selenium sulfide	7446-34-6		5	4	Dermal
Tetranitromethane	509-14-8		1	4	Inhalation
4,4′-Thiodianaline	139-65-1		7	11	Feed
2,6-Toluenediamine dihydrochloride	15481-70-6	+	2	0	Feed
2,5-Toluenediamine sulfate	6369-59-1		2	0	Feed
1,2,3-Trichloropropane	96-18-4		2	19	Gavage
2,4,5-Trimethylanaline	137-17-7		2	4	Feed
Trimethylphosphate	512-56-1		1	2	Gavage
4-Vinyl-1-cyclohexene diepoxide	106-87-6		1	5	Dermal
Ziram	137-30-4	+	1	2	Feed

Abbreviations: SAL, Ames *Salmonella* mutagenesis assay; CHO, Chinese hamster ovary cell assay for chromosomal abnormalities; CAS, Chemical Abstracts Registry number; CS, contact sensitization.

[a]RFB—primary rationale for the bioassay as follows: 1, widespread use; 2, structural relation with known carcinogen or mutagen; 3, inadequate previous study; 4, representative of a structural class; 5, drug with prolonged exposure; 6, potential use as an antineoplastic drug; 7, preexisting evidence of carcinogenicity; and 8, potential warfare agent.

[b]Tumor score is the number of different organs that showed tumor induction in mice and rats of both sexes; parentheses indicate the number of tumor sites that were obtained only on a single species; se-tumor responses indicate those that were classified as some evidence in contrast to the stronger designation of clear evidence.

about half of the contact sensitizers were positive in the cancer bioassay, supporting the possibility that contact sensitizers might be an important group of environmental carcinogens.

Results

The NTP technical reports were the only source of bioassay data used in this analysis, and only those chemicals that had both genotoxicity and carcinogenesis bioassays were used. The lists of chemicals were provided by the NTP on the basis of what had been completed and published as technical reports by May 1996; the chemicals were stratified according to the results of the Ames *Salmonella* assay (SAL) for mutagenicity and the Chinese hamster ovary cell (CHO) assay for chromosomal damage. The Ames assay was selected because it is regarded as the most predictive index of carcinogenicity (*8*). There were other tests of mutagenicity, e.g., on mammalian cells, that were not used consistently in the bioassay series and were not included in this analysis. The CHO assay was chosen on the possibility that contact sensitizers, by being interactive with protein, might cause genotoxicity by indirect mechanisms. Some of the chemicals had more repetitions of given genotoxicity tests than others.

Chemicals were identified as contact sensitizers by the use of a list of 384 such agents in one dermatology text (*6*) and confirmed by another (*9*), with the addition of parathion. There is no formally recognized registry of contact sensitizers with standardized inclusion criteria.

Tables 1–4 list the chemicals according to the four combinations of the two genotoxicity indices: SAL^+,CHO^+; SAL^+,CHO^-; SAL^-,CHO^+; and SAL^-,CHO^-. These tables indicate which chemicals are contact sensitizers, the rationale for their being selected for bioassay, and the route of exposure. About 90% of the routes of exposure were via the gastrointestinal tract in the total NTP sample: feed (56%), gavage (33%), and drinking water (2%). The other routes of exposure included intraperitoneal injection (1%), dermal application (2%), and inhalation (6%). There were no differences in the routes of exposure according to genotoxicity or contact sensitization.

Tumorigenicity is also indicated in Tables 1–4. Each chemical was effectively bioassayed four times with 50 animals each, i.e., in male and female rats and mice. We analyzed the data on the basis of the combined number of organs in the four test groups that had a statistically significant increase in tumor formation; the different tumor types within any given organ were lumped together, e.g., bile duct and hepatocellular tumors in the liver. The tumor

Table 2. SAL⁺,CHO⁺ NTP compounds

Chemical	CAS	CS	RFB[a]	Tumor score[b]	Route
Azobenzene	103-33-3		2	2	Feed
3-Amino-4-ethoxyacetanalide	17026-81-2		2	1	Feed
3-Amino-9-ethylcarbazole hydrochloride	132-32-1		2	8	Feed
3-Chloro-2-methylpropene	563-47-3		2	4	Gavage
C.I. Pigment red 23	6471-49-4		2	0	Feed
C.I. Pigment red 3	2425-85-6		2	4se	Feed
C.I. Acid red 114	6459-94-5		2	(11)	Water
C.I. Solvent yellow 14	842-07-9		7	2	Feed
C.I. Disperse yellow 3	2832-40-8	+	1	2se	Feed
C.I. Basic red 9	569-61-9		2	11	Feed
D & C Red No. 9	5160-02-1		1	2	Feed
p,p'-Ethyl DDD (perthane)	72-56-0		1	0	Feed
2,4-Diaminophenol dihydrochloride	137-09-7	+	2	1se	Gavage
Dimethylvinylchloride	513-37-1		2	11	Gavage
2,4-dinitrotoluene	121-14-2		2	2se	Feed
1,3-dichloropropene (Telone II)	542-75-6		2	6	Gavage
Dioxathion	78-34-2		1	0	Feed
Ethyl bromide (bromoethane)	74-96-4		2	2	Inhalation
Formulated fenaminosulf	140-56-7		2	0	Feed
HC Blue 2	33229-34-4		2	0	Feed
HC Yellow 4	59820-43-8		2	0	Feed
Iodoform	75-47-8		2	0	Gavage
Lead dimethyldithiocarbamate	19010-66-3		3	0	Feed
Methylene chloride	75-09-2		2	6	Inhalation
Methyl parathion	298-00-0		1	0	Feed
Nithiazide	139-94-6		1	3	Feed
4-Nitro-*o*-phenylenediamine	99-56-9		2	0	Feed
5-Nitro-*o*-anisidine	99-59-2		2	4	Feed
3-Nitro-*p*-acetophenetide	1777-84-0		2	1	Feed
Nitrofen	1836-75-5		1	2	Feed
Pentachloroanisole	1825-21-4		2	3se	Gavage
Photodieldrin	13366-73-9		2	0	Feed
2,4 and 2,5-Toluenediisocyanate	26471-62-5	+	1	8	Gavage
Tribromomethane	75-25-2		2	2	Gavage
Trifluralin	1582-09-8		1	3	Feed

Abbreviations: SAL, Ames *Salmonella* mutagenesis assay; CHO, Chinese hamster ovary cell assay for chromosomal abnormalities; CAS, Chemical Abstracts Registry number; CS, contact sensitization.
[a]RFB—primary rationale for the bioassay as follows: 1, widespread use; 2, structural relation with known carcinogen or mutagen; 3, inadequate previous study; 4, representative of a structural class; 5, drug with prolonged exposure; 6, potential use as an antineoplastic drug; 7, preexisting evidence of carcinogenicity; and 8, potential warfare agent.
[b]Tumor score is the number of different organs that showed tumor induction in mice and rats of both sexes; parentheses indicate the number of tumor sites that were obtained only on a single species; se-tumor responses indicate those that were classified as some evidence in contrast to the stronger designation of clear evidence.

response to each chemical in Tables 1–4 was the sum of the number of organs that had statistically significant tumor induction across all four test groups; this is called the tumor score. Thus, if the liver were the only organ with statistically significant tumor induction by a given chemical, and this occurred in male and female rats and mice, the tumor score for that chemical would be 4. If four different organs in female mice were involved in tumor induction and none of the three other groups showed a tumor response, the tumor score for that chemical would also be 4; this illustration is an extreme example of a non-uniform response, which in fact did not occur. The tumor score for each chemical was a measure of the pervasiveness of tumor induction with respect to sex, organ, and species. It was not a measure of potency in the sense of the daily dosage required to induce a given level of tumorigenic response. A zero score, of course, indicated a nontumorigenic response.

The terminology used in the technical reports changed over the years. The earlier reports used the terms positive or suggestive evidence, either of which we considered to be a tumorigenic response. The later reports used the terminology for the tumor bioassays as clear evidence, some evidence, equivocal evidence, or no evidence. We considered either clear or some evidence as an indication of tumorigenicity; the less decisive some evidence responses are indicated in Tables 1–4. The rationale for doing the bioassay was obtained from each technical report. The primary rationales are as follows: 1) widespread usage; 2) structural resemblance to a known carcinogen or mutagen; 3) an inadequate previous study; 4) representative of a structural class that had not been adequately studied; 5) a drug whose use is prolonged; 6) an antineoplastic drug; 7) preexisting evidence of carcinogenicity; and 8) potential use as a warfare agent. The first two rationales included 82% of the chemicals.

Table 5 summarizes the data from the entire NTP bioassay sample with respect to genotoxicity, contact sensitization, and tumorigenicity, including the proportion of some evidence tumor responses and the primary rationale for the decision to test the chemical. All of the differences, except where indicated, were statistically significant in a two-tailed test at $p = <0.05$. There was a total of 209 chemicals in the analysis, including the known contact sensitizers and those that were not known to be such. The majority of the chemicals were SAL⁺, i.e., 122 (58%) compared to 87 (42%) that were SAL⁻. In both the SAL⁺ and SAL⁻ groups, the majority of chemicals were CHO⁺, 71% and 74%, respectively.

The majority of the 209 tested chemicals were tumorigens (64%). Somewhat more of the SAL⁺ chemicals were tumorigenic (74%), compared to the SAL⁻ chemicals (51%). The 90 SAL⁺ tumorigens were more decisively tumorigenic in the sense that only 12% were in the some evidence category, compared to 34% for the 44 SAL⁻ tumorigens.

Somewhat more of the 209 chemicals were selected for bioassay because of structural resemblance to carcinogens or mutagens (Rationale 2; 47%), compared to those that were tested solely because of widespread use (Rationale 1; 35%). This was not statistically significant at $p = 0.05$. Comparing Rationales 1 and 2, the SAL⁺ group was heavily weighted (2.5:1) toward chemicals with structural resemblance to carcinogens (Rationale 2), while the SAL⁻ chemicals were heavily weighted (3.2:1) toward those that were selected because of widespread exposure (Rationale 1).

Of the 209 chemicals, there were 36 (17%) that were contact sensitizers for humans. Table 6 summarizes the data from the 36 contact sensitizing chemicals for the same parameters as those shown in Table 5 for the total sample of 209 chemicals. About two-thirds (63%) of the contact sensitizers were in the SAL⁻ category. This differs from the bioassay population as a whole, in which 42% were SAL⁻. The same high proportion of both SAL⁻ and SAL⁺ chemicals (80%) were CHO⁺ as in the entire bioassay sample (72%). As with the total sample of bioassay chemicals, the majority of the SAL⁺ sensitizers (55%) were tested because of structural resemblance to carcinogens and mutagens (Rationale 2), and most (80%) of the SAL⁻ sensitizers were bioassayed because of widespread usage (Rationale 1). About half (54%) of the contact sensitizers were tumorigenic; a larger proportion of the SAL⁺ contact sensitizers were tumorigenic (77%) compared to the SAL⁻ sensitizers

(41%). A larger proportion (44%) of the SAL⁻ tumorigens were in the some evidence category compared with 20% in the SAL⁺ sensitizer carcinogen group. Thus the contact sensitizers differed from the total sample of bioassay chemicals in only two respects: the rationale for selection was different and there was a greater proportion of SAL⁻ chemicals among the contact sensitizers.

Table 7 shows the frequency distribution of the tumor scores, i.e., the number of organs in which tumors were induced, as described above according to the Ames assay, for the total NTP sample and for contact sensitizers alone, without regard to the route of administration. The frequency distributions were biased toward the low tumor scores and were analyzed as log distributions. In the total NTP sample, the SAL⁺ chemicals had about twice the tumor score as the SAL⁻ chemicals, with a geometric mean and geometric standard deviation of 2.1 ± 2.3 and 0.9 ± 2.0, respectively. This was statistically significant ($p \geq 0.0001$). The SAL⁺ contact sensitizers also had a higher geometric mean tumor score than the SAL⁻ contact sensitizers, 1.3 ± 1.9 compared to 0.8 ± 2.1, but the difference was not statistically significant ($p = 0.3$). There were no blockbuster chemicals in any group except the SAL⁺, CHO⁺ group, in which 1,2-dibromoethane, 2,3-dibromo-1-propanol, glycidyl, and 1,2,3-trichloropropane had tumor scores of 15, 23, 25, and 19, respectively. There were a few SAL⁺ contact sensitizers that had substantial tumor scores such as coumarin (*9*), and 2,4-toluene diisocyanate (*9*). Among the SAL⁻ contact sensitizers, only *N*-methylolacrylamide and 2-mercaptobenzothiazole had substantial tumor scores of 7 and 6, respectively.

As indicated above, the characterization of tumor responses as tumor scores was a measure of the pervasiveness of the tumorigenic action across species, organs, and sex. Potency was characterized, with respect to genotoxicity, on the basis of the dosage (milligrams per kilogram per day) estimated to produce a 50% tumor incidence (TD_{50}) using published values (*10*) for many of the same chemicals that were included in this analysis but necessarily excluding those which were nontumorigenic. There were no statistically significant differences according to genotoxicity; this could hardly be otherwise because the range of potencies was enormous, as reflected by the extremes where the difference was a factor of 10^9.

There appeared to be a difference, according to genotoxicity, in the organs in which tumors were induced in both the total NTP sample and the contact sensitizers. The organs were stratified according to

Table 3. SAL⁻,CHO⁺ NTP compounds

Chemical	CAS	CS	RFB[a]	Tumor score[b]	Route
Acetominophen (4-hydroxyacetanilide)	103-90-2		1	0	Feed
Allyl isothiocyanate	57-06-7		1	1	Gavage
Allyl isovalerate	2835-39-4		1	2	Gavage
Anthranilic acid	118-92-3		1	0	Feed
Benzyl alcohol	100-51-6	+	1	0	Gavage
Gamma-butyrolactone	96-48-0		4	0	Gavage
Carbromol	77-65-6		2	0	Feed
D-Carvone	2244-16-8		1	(0)	Gavage
Chlorinated paraffins: C12, (60% chlorine)	63449-39-8		4	7	Gavage
2-Chloroacetophenone (CN)	532-27-4		1	0	Inhalation
3-Chloro-2-methylpropene	563-47-3		2	4	Gavage
Chlorothalonil	1897-45-64		1	2	Feed
4-Chloro-*o*-toluidine hydrochloride	3165-93-3		2	2	Feed
Chloropheniramine maleate	113-92-8		1	0	Gavage
C.I. Acid orange 10	1936-15-8		1	0	Feed
Diallyl phthalate	131-17-9		1	0	Gavage
Dibutyltin diacetate	1067-33-0		4	0	Feed
Di(2-ethylhexyl) adipate	103-23-1		1	2	Feed
N,N-dimethylaniline	121-69-7	+	2	1se	Gavage
Dimethyl morpholinophosphoramidate	597-25-1		8	2se	Gavage
Diphenhydramine hydrochloride	147-24-0	+	1	0	Feed
Ethyl acrylate	140-88-5	+	1	3	Gavage
Eugenol	97-53-0	+	2	0	Feed
Furan	110-00-9		1	8	Gavage
Furfural	98-01-1		1	3	Gavage
Furosemide	54-31-9		5	1se	Feed
Heptachlor	76-44-8		1	2	Feed
Hexachlorocyclopentadiene	77-47-4		2	0	Inhalation
Hydroquinone	123-31-9	+	1	3se	Gavage
Malathion	121-75-5	+	1	0	Feed
Manganese sulfate monohydrate	10034-96-5		3	0	Feed
2-Mercaptobenzothiazole	149-30-4	+	4	6se	Gavage
Mercuric chloride	7487-94-7	+	1	1se	Gavage
α-Methylbenzyl alcohol	98-85-1		2	1se	Gavage
Methyl methacrylate	80-62-6	+	2	0	Inhalation
N-methylolacrylamide	924-42-5	+	2	7	Gavage
Methylphenidate hydrochloride	298-59-9		5	2se	Feed
Monuron	150-68-5		3	2	Feed
Naphthalene	91-20-3		1	(1)se	Inhalation
p-Nitrophenol	100-02-7		1	0	Dermal
β-nitrostyrene	102-96-5		1	0	Gavage
Pentachloronitrobenzene	82-68-8	+	3	(0)	Feed
Pentachlorophenol, technical	87-86-5	+	1	(9)	Feed
Phenol	108-95-2		1	0	Water
Phenylbutazone	50-33-9		5	2se	Gavage
N-phenyl-*p*-phenylenediamine	101-54-2	+	2	0	Feed
1-Phenyl-2-thiourea	103-85-5		2	0	Feed
Picloram	1918-02-1		1	1se	Feed
Polysorbate 80 (glycol)	9005-65-6		1	0	Feed
Propyl gallate	121-79-9	+	1	0	Feed
Pyrimethamine	58-14-0		5	0	Feed
Resorcinol	108-46-3	+	1	0	Gavage
Rhodamine 6G	989-38-8	+	1	0	Feed
Sodium fluoride	7681-49-4		1	0	Water
Stannous chloride	7772-99-8		1	0	Feed
4,4′-Sulfonyldianaline (dapsone)	80-08-0		5	1	Feed
Tetraethylthiuram disufide	97-77-8	+	1	0	Feed
Tetrakis (hydroxymethyl) phosphonium chloride	124-64-1		2	0	Feed
Tetrakis (hydroxymethyl) phosphonium sulfide	55566-30-8		2	0	Feed
1,1,1-Trichloroethane	71-55-6		1	0	Gavage
1,1,2-Trichloroethane	79-00-5		1	2	Gavage
Turmeric oleoresin (curcumin)	8024-37-1		1	0	Feed
2,6-Xylidine	87-62-7		1	(4)	Feed
Zearalenone	17924-92-4		2	3	Feed

Abbreviations: SAL, Ames *Salmonella* mutagenesis assay; CHO, Chinese hamster ovary cell assay for chromosomal abnormalities; CAS, Chemical Abstracts Registry number; CS, contact sensitization.

[a]RFB— primary rationale for the bioassay as follows: 1, widespread use; 2, structural relation with known carcinogen or mutagen; 3, inadequate previous study; 4, representative of a structural class; 5, drug with prolonged exposure; 6, potential use as an antineoplastic drug; 7, preexisting evidence of carcinogenicity; and 8, potential warfare agent.

[b]Tumor score is the number of different organs that showed tumor induction in mice and rats of both sexes; parentheses indicate the number of tumor sites that were obtained only on a single species; se-tumor responses indicate those that were classified as some evidence in contrast to the stronger designation of clear evidence.

whether the tumors arose partially or entirely from epithelial lining or surface (mucosal) cells or mainly from parenchymal cells. The organs where tumors derived, at least partly, from lining epithelium included the mucosa of the bladder, the bronchial and nasal mucosae, the lining of the gastrointestinal tract from the mouth through the intestines, the skin and adnexal structures, the sebaceous gland of the ear canal (Zymbal gland), and the epithelia of the breast and clitoral and preputial glands. The parenchymal organs included the various tumors of the kidney, liver, endocrine glands, and the hemopoietic and lymphatic tissues. Table 8 presents the tumor scores for the indicated organs together with the corresponding percentage of the aggregate tumor scores for genotoxic and nongenotoxic chemicals. For example, the tumor score for the bladder among SAL$^+$ chemicals was 20, which was 5% of the aggregate tumor score of 389 for the SAL$^+$ chemicals in the total NTP sample; the combined subtotals are less than the aggregate totals because 14% of the tumors occurred in small numbers in miscellaneous organs and were not included in the analysis. This is true also for the SAL$^-$ chemicals in the total NTP sample and the contact sensitizers of both genotoxicity types. There was a predilection of SAL$^-$ chemicals for tumor induction in parenchymal tissues; i.e., 76% of tumors in the SAL$^-$ group were in parenchymal organs compared to 15% in epithelial organs; this was statistically significant at $p \leq 0.05$. In contrast, there was a roughly equal distribution of epithelial and parenchymal organ sites among the SAL$^+$ chemicals. Similarly, among the epithelial organ sites of tumor formation, there was approximately a threefold larger proportion of SAL$^+$ chemicals (49%) compared to SAL$^-$ chemicals (15%), whereas the opposite relationship was true for the parenchymal organs (37% and 76%, respectively). These comparisons were also statistically significant at $p \leq 0.05$. Roughly the same pattern was observed with the contact sensitizers as with the NTP sample as a whole, but without statistical significance. The much larger aggregate tumor score for the SAL$^+$ chemicals (389), compared to the SAL$^-$ chemicals (115) was due to the larger number of SAL$^+$ chemicals, the higher proportion of tumor-responding animals, and the larger number of organs in which tumors were induced.

The relative inability of SAL$^-$ chemicals to induce tumors in epithelial tissues raises the question of whether this might be due to differences in background tumor rates; i.e., if nongenotoxic chemicals are only tumor promoters and tumor promoters act by accelerating tumorigenic processes that are under way, they would be expected to have a predilection for organ sites with relatively high background tumor rates. There was a correlation between tumor induction and background tumor rates in the sense that, when a chemical produced a larger

Table 4. SAL$^-$,CHO$^-$ NTP compounds

Chemical	CAS	CS	RFB[a]	Tumor score[b]	Route
11-Aminoundecanoic acid	2432-99-7		1	1	Feed
Benzoin	119-53-9	+	1	0	Feed
Benzyl acetate	140-11-4		1	5	Gavage
Calcium cyanamide	156-62-7		3	0	Feed
Chloroform	67-66-3		2	3	Gavage
Cinnamyl anthranilate	87-29-6		3	4	Feed
p-Dichlorobenzene	106-46-7	+	1	3	Gavage
1,2-Dichlorobenzene	95-50-1	+	1	0	Gavage
Di(2-ethylhexyl) phthalate	117-81-7	+	1	4	Feed
5,5-Diphenylhydantoin	57-41-0		5	1	Feed
Ethylene thiourea	96-45-7		1	8	Feed
Fenthion	55-38-9		1	1se	Feed
FD&C Yellow No. 6	2783-94-0		1	0	Feed
Isophorone	78-59-1		1	2se	Gavage
Maloxon	1634-76-2		1	0	Feed
Parathion	56-38-2	+	1	2se	Feed
Pentachloroethane	76-01-7		2	2	Gavage
Sulfisoxazole	127-69-5		5	0	Gavage
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	1746-01-6		1	5	Gavage
Titanium dioxide	13463-67-7		1	0	Feed
Trichloroethylene	79-01-6		2	(2)	Gavage
Tetrachloroethylene	127-18-4		2	5	Inhalation
Tris(2-ethylhexyl) phosphate	78-48-2		1	1se	Gavage

Abbreviations: SAL, Ames *Salmonella* mutagenesis assay; CHO, Chinese hamster ovary cell assay for chromosomal abnormalities; CAS, Chemical Abstracts Registry number; CS, contact sensitization.
[a]RFB—primary rationale for the bioassay as follows: 1, widespread use; 2, structural relation with known carcinogen or mutagen; 3, inadequate previous study; 4, representative of a structural class; 5, drug with prolonged exposure; 6, potential use as an antineoplastic drug; 7, preexisting evidence of carcinogenicity; and 8, potential warfare agent.
[b]Tumor score is the number of different organs that showed tumor induction in mice and rats of both sexes; parentheses indicate the number of tumor sites that were obtained only on a single species; se-tumor responses indicate those that were classified as some evidence in contrast to the stronger designation of clear evidence.

Table 5. Various characteristics of the total NTP bioassay sample according to patterns of genotoxicity

	SAL$^+$,CHO$^+$		SAL$^+$,CHO$^-$		SAL$^+$		SAL$^-$,CHO$^+$		SAL$^-$,CHO$^-$		SAL$^-$		Total	
Genotoxicity	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Chemicals (*n*)	87	(100)	35	(100)	122	(100)	64	(100)	23	(100)	87	(100)	209	(100)
		42→		17→		58→		31→		11→		42→		
Tumorigens	67	77↑	24	69↑	91	75↑	29	45↑	16	70↑	45	52↑	136	65↑
		50→		17→		67→		21→		12→		33→		
Tumorigens, some evidence[a]	06	09↑	05	21↑	11	12↑	11	38↑	04	25↑	15	33↑	26	19↑
		23→		19→		42→		42→		15→		58→		
Rationale 1	12	14↑	09	26↑	21	17↑	37	58↑	15	65↑	52	60↑	73	35↑
		16→		12→		29→		51→		21→		71→		
Rationale 2	58	67↑	24	69↑	82	67↑	14	22↑	04	17↑	18	21↑	100	48↑
		58→		24→		82→		14→		04→		18→		
Other rationale	17	20↑	02	06↑	19	16↑	13	20↑	04	17↑	17	20↑	36	17↑
		47→		06→		53→		36→		11→		47→		

Abbreviations: SAL, Ames *Salmonella* mutagenesis assay; CHO, Chinese hamster ovary cell assay for chromosomal abnormalties. Each vertical arrow refers to the percentage of the number at the top of the same column. Each horizontal arrow refers to the percentage of the number in the right hand box in the same row.
[a]Percentage of tumorigens that show some evidence.

Table 6. Characteristics of the contact sensitizers with respect to patterns of genotoxicity

	SAL^+,CHO^+		SAL^+,CHO^-	SAL^+		SAL^-,CHO^+		SAL^-,CHO^-	SAL^-		Total	
Genotoxicity	Number	Percent	Number	Number	Percent	Number	Percent	Number	Number	Percent	Number	Percent
Chemicals (*n*)	10	(100)	03	13	(100) 36→	18	(100)	05	23	(100) 64→	36	(100) (100)
Tumorigens	07	70↑	03	10	77↑ 50→	07	39↑	03	10	43↑ 50→	20	56↑ (100)
Tumorigens, some evidence[a]	01	14↑	02	03	30↑ 37→	04	57↑	01	05	50↑ 63→	08	40↑ (100)
Rationale 1	02	20↑	02	04	31↑ 20→	11	61↑	05	16	70↑ 80→	20	56↑ (100)
Rationale 2	06	60↑	0	06	46↑ 55→	05	28↑	0	05	22↑ 45→	11	31↑ (100)

Abbreviations: SAL, Ames *Salmonella* mutagenesis assay; CHO, Chinese hamster ovary cell assay for chromosomal abnormalties. Each vertical arrow refers to the percentage of the number at the top of the same column. Each horizontal arrow refers to the percentage of the number in the right hand box in the same row.
[a]Percentage of tumorigens that show some evidence.

tumor response in a given organ in one species compared to another, the background tumor rate was generally higher in the organ of the species with the higher tumor response. Table 9 compares the background tumor rates in various organs of rats and mice (*11*) with the tumor scores for individual organs for the 209 chemicals in the total NTP samples. Only those organs that had a minimum tumor score of 5 are included in the table. For example, in Table 9, the tumor scores for the rat and mouse livers were 45 and 99, respectively, and the corresponding background rates for males and females combined were 4 and 62%, respectively, showing a concordance between the species predominance of liver tumor induction and background tumor rates. Of the 20 organ comparisons, 15 (75%) showed a concordance between the higher species response and the higher background tumor incidence.

While there is an association of background tumor rates and species responses, some of the organs that had epithelial tumors also had high background tumor rates that were not enhanced by SAL^- chemicals, such as the lung in mice and the mammary gland in rats. Hence the explanation for the differences in organ patterns of tumor formation according to genotoxicity is not likely to be simple.

To evaluate the frequency of occurrence of contact sensitizers among chemicals in general, we searched the 10,000 entries in the *Merck Index* (*12*) for contact sensitizers. We made four passes through the chemicals by taking every hundredth chemical and comparing it to the list of contact sensitizers used above, starting with 1, 101, 1001, 9901; 20, 120, 1020, 9920; 50, 150, 1050, 9950; and 70, 170, 1070, 9970. In each of the four passes of about 100 chemicals each, we identified 4,4,2, and 2 contact sensitizers, respectively, for an average frequency of 3% [standard deviation (SD) = 1%].

Table 7. The number and percent (in parentheses) of bioassay chemicals that induced tumors in the indicated number of organs (tumor scores) according to genotoxicity for the total NTP bioassay sample and for the contact sensitizers alone

	Total NTP bioassays		Contact sensitizers	
Tumor scores	SAL^+	SAL^-	SAL^+	SAL^-
0	30 (26)	40 (49)	3 (23)	12 (57)
1–3	43 (37)	30 (37)	8 (62)	6 (29)
4–9	34 (29)	11 (14)	2 (15)	3 (14)
≥10	9 (8)	0 (0)	0 (0)	0 (0)
Chemicals	116 (100)	81 (100)	13 (100)	21 (100)
Geometric mean (SD)[a]	2.1 (2.3)	0.9 (2.0)	1.3 (1.9)	0.8 (2.1)

SAL, Ames *Salmonella* assay. All bioassays used were done on both rats and mice.
[a]SD is geometric standard deviation.

Table 8. Tumor induction sites, according to genotoxicity

	Total NTP sample		Contact sensitizers	
Organs	SAL^+	SAL^-	SAL^+	SAL^-
Organs in which tumors arose in part or entirely from epithelial cells				
Bladder	20 (5)	2 (2)	2 (10)	0 (0)
Lung/nose	46 (12)	4 (3)	3 (14)	2 (7)
Gastrointestinal[a]	63 (16)	8 (7)	1 (5)	2 (7)
Skin/zymbal	40 (10)	0 (0)	0 (0)	0 (0)
Reproductive[b]	25 (6)	3 (3)	1 (5)	0 (0)
Subtotal	194 (49)	17 (15)	7 (34)	4 (14)
Organs in which tumors arose mainly from parenchymal cells				
Kidney	9 (2)	10 (9)	2 (10)	2 (7)
Leukemia/lymphoma	16 (4)	8 (7)	0 (0)	2 (7)
Liver	92 (24)	51 (44)	6 (29)	11 (38)
Endocrine[c]	28 (7)	18 (16)	2 (10)	4 (14)
Subtotal	145 (37)	87 (76)	10 (49)	19 (66)
Aggregate tumor scores[d]	389 (86)	115 (91)	21 (83)	29 (80)

SAL, Ames *Salmonella* assay. Values shown are tumor score for the indicated organ, with the proportion of the total tumor score shown in parentheses.
[a]Includes oral, tongue, esophagus, stomach, and intestine.
[b]Includes mammary, clitoral, and preputial glands.
[c]Includes thyroid, adrenal, and pituitary.
[d]The aggregate percentage is less than 100% because miscellaneous organs in which small numbers of tumors occurred were not included in the analysis.

Discussion

Summarizing the above findings, it can be said that of the 209 bioassayed chemicals included in this analysis, 36 were known to be human contact sensitizers (17%). The contact sensitizers differed from the NTP bioassay group as a whole in that a larger proportion were nongenotoxic, which was probably related to the fact that fewer of them were selected for bioassay on the basis of their structural resemblance to known carcinogens, in contrast to widespread usage. When sorted for genotoxicity, the

Table 9. Comparison of rats and mice of both sexes in terms of the tumor scores in the indicated organs in relation to the background tumor rates

	Tumor scores		Background tumor incidence (%)[a]		
Organs	Rat	Mouse	Rat	Mouse	Concordance
Bladder*	18	4	0.6	0.6	No
Kidney *	20	1	1.0	0.5	Yes
Leukemia*	17	2	77	0.1	Yes
Lymphoma	2	3	1	34	No
Oral/esophagus*	11	1	1.9	0.1	Yes
Forestomach	28	23	0.3	3.3	No
Intestine*	10	0	0.6	1.4	No
Liver*	45	99	4	62	Yes
Pancreas*	7	0	1.9	0.1	Yes
Thyroid	17	13	31	4	Yes
Adrenal	12	8	38	6	Yes
Pituitary*	1	6	83	16	No
Vascular*	2	11	1	10	Yes
Sarcoma (all)	11	6	10	9	No
Skin epidermal and adnexa*	19	6	7	0.3	Yes
Zymbal*	18	1	1.5	0.1	Yes
Mammary*	16	4	48	1.3	Yes
Ovary	0	2	1.0	1.0	No
Uterus	3	4	14	3	No
Clitoral/preputial*	11	1	22	0.1	Yes
Nose*	14	6	0.8	0.1	Yes
Lung*	8	23	5	28	Yes
Harderian gland*	2	10	0	9	Yes
Total tumor score	286	234			
Number of animals	198	195			

[a]Background incidences of the sexes is summed.
*Difference between rat and mouse tumor responses are statistically significant ($p \leq 0.05$).

contact sensitizers had about the same characteristics as the total population of bioassayed chemicals in the following respects: 1) roughly the same proportion were tumorigenic; 2) proportionately more of the SAL^+ chemicals were tumorigenic than those that were SAL^-; 3) the SAL^- tumorigens had a greater proportion of less decisive (some evidence) tumor responses than the SAL^+ tumorigens; 4) the tumor scores were greater in the SAL^+ chemicals than in the SAL^- chemicals; and 5) the SAL^- chemicals had proportionately more parenchymal tumors (renal, liver, and endocrine tumors and leukemia) than the SAL^+ chemicals, whereas the latter produced relatively more epithelial tumors [bladder, gastrointestinal, skin and zymbal gland, breast, clitoral and preputial glands, and respiratory tumors (nose and lung)].

Most of the chemicals were CHO^+ regardless of whether they were SAL^+ or SAL^- or whether they were carcinogens. This may reflect the possibility that the underlying reason why all of the chemicals were chosen for bioassay, regardless of the specific rationale, was because they were highly reactive as intermediates in chemical synthesis or as reactive finished products; perhaps highly reactive chemicals tend to react with protein. This might mean that many of the NTP bioassay chemicals are contact sensitizers. If so, a comparison of the characteristics of the contact sensitizers to the total group of bioassay chemicals has the limitation that some substantial fraction of the chemicals that were not known to be contact sensitizers may actually be so.

The concentration of contact sensitizers in the $SAL^- CHO^+$ category is unexplained. The status of a chemical as CHO^+ or CHO^- did not consistently affect tumorigenicity. The $SAL^- CHO^-$ group was anomalous because of its high proportion of tumorigens.

The similarity of the characteristics of the contact sensitizers with those of the entire NTP sample raises the issue of whether contact sensitization might be unrelated to tumorigenicity, i.e., selecting for contact sensitizers constitutes a random sample of bioassay chemicals. However, contact sensitizers were not a random sample because of the differences in the rationale for selection; the properties of the contact sensitizers and the total NTP sample only matched when the two groups were compared on the basis of mutagenicity.

The connection between tumorigens and contact sensitizers relates to the ability of both classes of chemicals to interact with protein. The contact sensitizers react with the amino acid side chains of proteins by a variety of mechanisms: two electron reactions, including nucleophilic and electrophilic substitutions and additions, as well as single electron (free radical) additions (*2*). Genotoxic tumorigens, as electrophiles, undergo the same kinds of interactions with proteins as contact sensitizers, but in the case of genotoxic tumorigens, the electrophilic interactions extend to DNA. There are subtle differences in chemical reactivity that depend on the strength of the electrophilic and nucleophilic centers, as well as the stearic properties of these chemicals that affect the amount and location of the adducts on protein and DNA. Also, there are important biological factors that have an effect on the outcome of the chemical reactions of carcinogens and contact sensitizers. When an electrophile, a hapten in the context of ACD, is adducted onto the amino acid of a protein, it may become an antigen if the resultant complex has a structure that is capable of sensitizing T lymphocytes; this is a biological response that can have a great deal of individual variability among humans. When an electrophile is adducted onto DNA, it may result in an oncogenic mutation as a consequence of mistakes in DNA repair processes. Hence, even though there is a commonality in the chemical behavior of contact sensitizers and genotoxic tumorigens as electrophiles, there are strong chemical and biological factors that can modulate their effects. However, there is sufficient overlap between the actions of contact sensitizers and tumorigens to raise the possibility that when a chemical is identified as a contact sensitizer, it has a significant likelihood of being a tumorigen.

ACD, caused by SAL^- contact sensitizers, might be a useful biomarker for identifying nongenotoxic tumor promoters. SAL^+ contact sensitizers would be expected to be both tumor promoters and initiators. A possible common denominator between contact sensitization and tumor promotion might be the induction of inflammation. ACD is an inflammatory reaction caused by a complex immune response set off by the chemical interaction of the sensitizer, the haptene, with skin proteins. The resultant complex, the antigen, ultimately sensitizes T lymphocytes, which are drawn to the site of skin exposure where they induce a Type IV cell-mediated inflammatory reaction. Contact sensitization also occurs in animals, and the inflammatory reaction is indistinguishable from inflammation produced by skin tumor promoters. Inflammation is a characteristic of many tumor promoters (*13,14*). Mechanistically, the connection between inflammation and tumor promotion is thought to involve the production of free radicals (*15,16*). Some tumor promoters are contact sensitizers, including benzoyl peroxide (*17*), phorbol

myristate acetate (*1*), and 2,4-dinitro-1-fluorobenzene (*1*) for mouse skin and butyl hydroxytoluene for rat liver (*18*). The ability of tumorigens to induce inflammation may be the basis for the promoting component of their action; whether this is due to an immune or irritant reaction is not known (*19*).

The action of contact sensitizing tumorigens may be stimulated by the induction of humoral immunity. For example, mice injected with rabbit benzo(*a*)pyrene (BaP) antibodies show a much enhanced skin tumor response to BaP applied dermally (*20*). Moreover, there is evidence that transplanted malignant cells grow faster in a host that had been previously immunized against them (*21*).

We examined the issue of whether contact sensitizers are tumor promoters as a consequence of their immunological effects in a previous study that used a well-known contact sensitizer and tumor promoter (*22,23*), 1-fluoro 2,4-dinitrobenzene (DNFB), in Tg.AC mouse skin. This transgenic mouse is tumor initiated by the presence of a mutated *ras* oncogene and is therefore a model for skin tumor promotion (*24*). The skin application of DNFB produced the typical promotional response of squamous papillomas, with an associated dermal inflammatory reaction. However, the immune-depressing corticosteroid fluocinolone acetonide applied locally did not reduce either the inflammation or the tumor response, suggesting that some component of cytotoxicity, not immunogenicity, caused both the inflammation and the tumor promotion, namely, that contact sensitization and tumor promotion may be independent responses to electrophilic chemicals (*1*).

Figure 1 illustrates some of the interrelationships that might exist between electrophilicity, macromolecular adduction, and various biological responses. Two linkages of electrophilicity are shown: DNA and protein adduction. DNA adduction is linked to mutagenicity and thus to tumor initiation and progression. Protein adduction is shown to have a four-way linkage to cell-mediated immunity (contact sensitization), humoral immunity, cytotoxicity, and indirectly to mutagenicity (e.g., possibly by interference with DNA repair and chromosomal segregation). The immune and cytotoxic responses are linked with inflammation and thus with tumor promotion. Promotion of atherosclerotic lesions is included on the basis that polycyclic aromatic hydrocarbon carcinogens like benzo(*a*)pyrene and 7,12 dimethylbenz(*a*)anthracene promote the development of aortic atheromata in chickens (*25*); this finding was prompted by the evidence

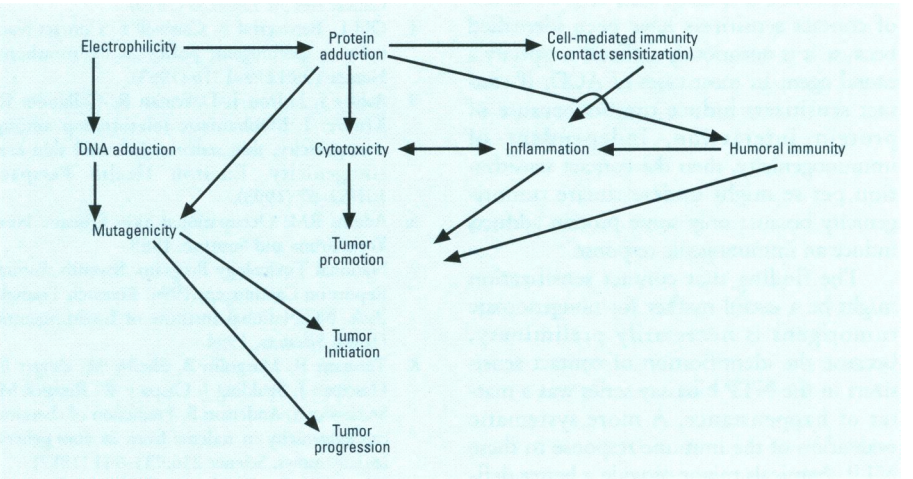

Figure 1. Possible interrelationships between electrophilicity, macromolecular adduction, and various biological responses.

that aortic atheromatous plaques in humans have a tumorlike monoclonal origin (*26*). The evidence for the existence of some of these linkages is modest. However, Figure 1 does illustrate that the contact sensitizing and carcinogenic properties of electrophilic chemicals could be independent expressions of their common underlying chemical (electrophilic) reactions, i.e., that contact sensitizers are not tumorigens on the basis of their immunogenic effects but rather because of some aspect of their toxicity. It would be useful to know whether the immune response of contact sensitizers enhances tumorigenicity; if so, immunogenically responsive people might be at increased tumorigenic risk.

Carcinogen-induced deletion of proteins, vital to growth control, was a prominent theory of cancer that stemmed from early work on protein binding in the liver by *p*-aminoazobenzene, a chemical that, interestingly, is a contact sensitizer (*27*). This theory was overshadowed by the discovery of carcinogen-induced genotoxicity and was relegated to an epigenetic role (*3*). Perhaps the epigenetic role of electrophile-induced protein damage is promotion of genetic damage.

A combination of the Ames assay for mutagenicity and an assay for contact sensitization, as a screening tool, might be an improvement in the detection efficiency for environmental tumorigens over that from the Ames assay alone because it would help to uncover rodent tumorigens among the SAL^- chemicals. This is seen in an analysis of the Rationale 1 chemicals (widespread use without structural resemblance to known carcinogens), the group that is probably the most relevant to the generality of chemicals in the environment. Of the 73 Rationale 1 chemicals, 23 were SAL^+ (32%); of these, 15 were tumorigens on bioassay (65%) with an overall tumorigenic detection efficiency of 21% (15/73). Of the remaining 50 chemicals that were SAL^-, 16 (32%) were contact sensitizers; of these, 7 (44%) were tumorigenic on bioassay for an overall detection efficiency of 14% (7/50). Thus, in this illustration, if a contact sensitization bioassay followed the Ames test, the yield of rodent tumorigens among the 73 Category I chemicals would rise from 15 to 22, a 43% increase because of the inclusion of SAL^- chemicals. An evaluation of false negatives cannot be made because the unknown proportion of chemicals that are not recognized as contact sensitizers, but might be if they were tested. There would be little or no advantage to combining the Ames assay with an assay for contact sensitization when the Ames test is positive because there is a statistically insignificant increase in the efficiency of detecting tumorigens; i.e., 77% compared to 65%.

The 3% of *Merck Index* chemicals that are contact sensitizers contrasts with the 17% of chemicals in the NTP bioassay series. The Merck chemicals were selected because they were biologically related, and the NTP chemicals were mostly reactive industrial intermediates or compounds in commercial use because of their pesticidal toxicity. The proportion of the 60,000 chemicals in commercial use (*28*) that are contact sensitizers ranges from 3% to 17%. An average of 10%, for purposes of estimation, yields perhaps 6,000 contact sensitizers in commercial use. If, as in this analysis of the NTP experience, about half of the contact sensitizers are tumorigenic in the rodent bioassay, there would be about 3,000 chemicals in commerce that would have to be taken seriously as possible tumorigens.

Furthermore, it is likely that only a fraction of contact sensitizers have been identified because it is notoriously difficult to specify a causal agent in most cases of ACD. If contact sensitizers induce tumors because of protein interaction, independent of immunogenicity, then the contact sensitization per se might underestimate tumorigenicity because only some protein adducts induce an immunogenic response.

The finding that contact sensitization might be a useful marker for nongenotoxic tumorigens is necessarily preliminary, because the identification of contact sensitizers in the NTP bioassay series was a matter of happenstance. A more systematic evaluation of the immune response to these NTP chemicals might provide a better definition of the utility of contact sensitization as a screen for potential nongenotoxic environmental tumorigens. An improved understanding of the nature of the macromolecular interactions of contact sensitizers might elucidate why some of these electrophiles are protein interactive but nongenotoxic; also, a better understanding of the chemical mechanisms might lead to an *in vitro* test that is more relevant to tumorigenesis than contact sensitization.

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